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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	WILBUR, D.S.	ART UNIT: 1617
SERIAL NO.:	09/750,280	EXAMINER: S. KANTAMNENI
FILED:	DECEMBER 29, 2000	CONF. NO.: 6495
FOR:	TRIFUNCTIONAL REAGENT FOR CONJUGATION TO A BIOMOLECULE	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

Dear Sir:

I, D. Scott Wilbur, Ph.D., hereby state that I am a co-inventor of the referenced application. I began my career after receiving a Ph.D. in synthetic organic chemist from the University of California at Irvine nearly 28 years ago. My first position was in the Medical Radioisotope Research group at the Los Alamos National Laboratory in Los Alamos, New Mexico. For six years in that position I conducted many synthetic chemistry studies in that position and published a number of papers. My second position was in a biotechnology start-up company in Seattle, Washington focused on derivatized monoclonal antibodies for diagnosis and therapy. There, I also conducted many synthetic chemistry studies over the next six years. Some 16 years ago I moved to the University of Washington to work. Over the past 15 years I have conducted studies with biotin derivatives in the development of new drugs for cancer treatment. In those studies it was discovered that biotin compounds must be derivatized in a manner such that they contain biotinidase-blocking moieties. When I started those studies there were only a few references in the literature that mentioned biotinidase. When I discovered that the radiolabeled biotin derivatives that were injected into mice were being degraded rapidly, I felt I had to study the effect of derivatization of biotin compounds to block their

degradation by biotinidase. My first paper on that subject was published in *Bioconjugate Chemistry 5*, 572-584, 1997. Although there were more references to biotinidase cleavage in the interim years, I still felt that the data was insufficient to allow researchers to know what functionality blocked biotinidase. So, further studies were conducted and I published a second paper on the subject in *Bioconjugate Chemistry 12*, 616-623, 2001. While those studies again demonstrated that certain functional groups alpha to the biotinamide bond would completely block the action of biotinidase, its actual mechanism was not clear, leaving one with a question of whether a functional group other than the ones defined would work. More recently, I have conducted further studies to define whether it was steric hindrance about the biotinamide bond that resulted in biotinidase blockage. Those studies are completed and a manuscript has been submitted to Bioconjugate Chemistry showing that it is steric hindrance, and that a very specific size of group must be used to balance blockage of the biotinidase with retention of the biotin binding with avidin or streptavidin required for in vivo function.

1. In regard to the referenced application and the Office Action of March 14, 2006, I understand that there is a rejection of claim 108 as being obvious over the combination of Rosebrough, S. (1993) J. Pharmacol Exptl. Therapeutics 265(1):408, and Wilbur, S. et al., WO 97/29114. I understand that Wilbur et al., WO 97/29114 is acknowledged by the Examiner as not teaching the conjugate of an aspartyl moiety and biotin as in claim 108, and Rosebrough is purported to teach a cysteinyl group conjugated to biotin, resulting in a carboxy group placed alpha to the amide bond, which is the target cleavage site for biotinidase. I understand that the Patent Office maintains that Rosebrough in combination with Wilbur et al. suggests the aspartyl moiety and/or the beta placement of a carboxy residue relative to the target amide group for biotinidase. While neither Wilbur et al. nor Rosebrough teach the aspartyl moiety or beta placement of the carboxy relative to the amide group, the Patent Office argues that since there is only a single methylene difference in carboxy moiety placement, use of the aspartyl residue in place of cysteine would be obvious.

WO 97/29114 mentions many possible "steric moieties" at the alpha position relative to the amide bond between the linker and the biotin. In 1997, the concept of

"steric moieties" was a hypothesis that had not been tested. In the paragraph spanning pages 17 and 18 of WO 97/29114, the preferred steric group is a methyl group alpha to the amide bond. In fact, through testing (and described in our publication), it was found that the methyl group slows biotinidase cleavage, but does not block it. Therefore, the concept of steric groups may be valid, but the size required was not known until only recently. While other steric groups are possible, WO 97/29114 states that such groups can adversely impact affinity of the biotin for avidin or streptavidin, which would undermine the purpose of forming the biotin-linker conjugate.

Rosebrough describes a comparative study of deferobiotin (DB), defero-desaminolysyl-biotin (DLB) and defero-acetyl-cysteinyl-biotin (DACB). Deferoxamine is a chelator that is useful in binding to radionuclides useful in diagnosis and treatment. One purpose of the study was to determine if placement of a carboxy group alpha to the amide linkage between biotin and the desferoxamine (in the DACB compound) would reduce catalysis by biotinidase (see page 413, first column, paragraph 1). It was in fact found that biotinidase metabolism of DACB by biotinidase was significantly reduced relative to DB and DLB (page 413, first column, paragraph 2; and column 2, paragraph 1). However, Rosebrough does not test or speculate about other biotin derivatives with a carboxy residue at the beta position or about a substitution of an aspartyl for a cysteinyl residue. Further, Rosebrough did not define whether the combination of the cysteine and deferoxime with biotin was required to block biotinidase, making it impossible to predict whether other biotin derivatives would also be blocked from biotinidase when the cysteine moiety was used.

The compounds of claim 108 would not be considered by a scientist in the field to contain homologues of the Rosebrough DACB compound as the DACB conjugate is a cysteinyl-biotin conjugate, whereas the claim 108 compound is an aspartyl-biotin conjugate. In addition to the insertion of a methylene group (as mentioned by the Examiner), the claim 108 compounds also delete or replace the –S- of the cysteinyl with a carboxyl or carbonyl group. According to Lewis, R. (ed.) Hawley's Condensed Chemical Dictionary (12th ed. 1993), Van Nostrand Reinhold, p. 606, a "homologous series" is "a series of organic compounds in which each successive member has one more CH_2 group

in its molecule than the preceding member." Homologues do not seem to encompass compounds where -S- has been replaced by a carboxy or carbonyl group.

2. In 2000, the year the subject application was filed, few structure/function studies on biotinidase had been conducted. Swango et al. (2000) Mol. Genet. Metab. 69:111-115, reported on homologies among human biotinidases, bacterial aliphatic amidases and bacterial and plant nitrilases. Each of these enzymes is a hydrolase that cleaves C-N bonds other than peptide bonds. The authors suggested that the catalytic mechanisms among these three enzymes were similar, and identify the $YRK_{210-212}$ region as involved in the active site, and speculate that Cys_{245} may also be involved in the active site. In 2000, no X-ray crystal structure or other model of biotinidase catalytic and/or binding sites was available. Thus, it would not have been possible to reasonably or reliably predict that certain substitutions located beta to the target amide bond would or would not have inhibited biotinidase activity without conducting an experiment using the contemplated substitution.

As of 2005, it had been found that biotinidase from mammals, insects and fungi demonstrated considerable homology. Among mammals, the amino acid sequence was reported to be highly conserved. Several motifs were found to be conserved among animals. It has been reported that "three highly conserved regions are likely to be involved in the active site of the enzyme and are essential for catalysis of the amide linkage" of biocytin (Wolf et al. (2005) Mol. Genet Metab 86:44050; at page 45, column 2, paragraph 3; page 46, column 2, paragraph 2; page 47, column 1, first paragraph). Wolf et al. speculated that the biocytin/biotin binding sequence of biotinidase is in the last third of the protein. Highly conserved positions such as Cys residues at 424, 458, 463 and 471, as well as highly conserved residues at 438-441 and 451-454 "may be important for biocytin/biotin binding and enzymatic function" (page 47, first column, paragraph 3). At page 49, first column, paragraph 1, Wolf et al. stated that *because biotinidase has not been successfully crystallized, its tertiary structure has not been defined.* This indicates that mechanisms of catalytic activity and/or binding had not been fully defined even in 2005.

From Wolf et al., it is clear that, as of 2005, persons in the field still could not reasonably predict alterations near the amide bond of biocytin or biotin-linker conjugates that would inhibit biotinidase activity, unless experiments with the contemplated alterations had already been or were conducted.

Respectfully submitted,

9/12/06
Date

D. Scott Wilbur, Ph.D.

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